

IN THE SPECIFICATION:

Please amend the specification as noted.

1. Please replace existing paragraph **0003** with the following paragraph:

--Tuberculosis (TB) is the primary cause of death due to a single microbial pathogen, accounting for 2 to 3 million deaths and an estimated 8 to 10 million new cases per year worldwide (1). It is estimated that one third of the world's population is infected with *Mycobacterium tuberculosis*.

The global epidemic is growing, concomitant with HIV co-infection and the increasing prevalence of drug resistant strains. Genotyping clinical isolates of ~~M:~~ *M. tuberculosis* has the potential to significantly impact both clinical practice and public health. Clinically, determining strain relationships can distinguish treatment failure versus potential laboratory cross-contamination, and reactivation of drug-resistant disease versus infection with another strain (24). In the public health arena, determining strain relationships during the course of a tuberculosis outbreak can focus public health resources. However, the most discriminatory ~~M:~~ *M. tuberculosis* genotyping method, IS6110 restriction fragment length polymorphism (RFLP) analysis, is time-consuming, technically demanding, and difficult to standardize between laboratories (3). In addition, IS6110 RFLP does not provide sufficient strain discrimination when fewer than six IS6110-hybridizing bands are present. A secondary genotyping method is required in these instances. A number of PCR-based genotyping methods have been developed, but are limited in their ability to reproducibly provide a level of strain discrimination that matches associations defined by traditional epidemiology. In addition, the high degree of coding region sequence conservation between strains of ~~M:~~ *M. tuberculosis* virtually excludes the use of more commonly used population genetics methods like multi-locus enzyme electrophoresis, restriction fragment length end labeling analysis, and gene sequencing (12).--

2. Please replace existing paragraph **0013** with the following paragraph:

--In another important aspect of the invention, primer pairs comprising a forward and a reverse primer are presented for amplification of VNTR located in DNA from a *M. tuberculosis* species. Primer pairs suitable for PCR amplification of VNTR, by MLVA or by multiplex, may be selected from the group consisting of SEQ ID NO: 1 and 2, SEQ ID NO: 3 and 4, SEQ ID

NO: 5 and 6, SEQ ID NO: 7 and 8, SEQ ID NO: 9 and 10, SEQ ID NO: 11 and 12, SEQ ID NO: 13 and 14, SEQ ID NO: 15 and 16, and SEQ ID NO: 17 and 18. Certain preferred primer pairs have, in addition, an observable group whereby amplified product may be detected. Such groups may be, for example, a fluorescent group or a radioactive group.--

3. Please replace existing paragraph **0020** with the following paragraph:

--The ultimate utility of VNTR loci lies in their diversity. The present invention discloses the use of marker diversity using both allele number and frequency to sub-type *Mycobacterium tuberculosis* species. VNTR markers that exhibit high diversity values possess great discriminatory capacity for identifying genetically similar strains. Less diverse markers may be applied with greater utility in species identification and the analysis of evolutionary relationships. This demonstrated ability to predict VNTR diversity based upon array size allows the guided selection of marker loci.--

4. Please replace existing paragraph **0058** with the following paragraph:

--The complete genome sequence of the *Mycobacterium tuberculosis* H37Rv strain was downloaded from the Sanger Centre website (www.sanger.ac.uk). Potentially polymorphic repetitive sequences were identified using the DNASTAR software program Genequest (Lasergene, Inc Madison, WI). Selection criteria of repetitive sequence were set for nucleotide repeat motifs of more than 8bp within 100 bp proximity of each other. Primers were designed around 84 repetitive sequences identified using the DNASTAR software program Primer Select (Lasergene, Inc Madison, WA). Complementary primers were designed around interspersed repeats to minimize risk of mobile DNA targets. Fifteen of these repetitive sequences were found to be polymorphic, i.e., VNTR loci. Nine loci were chosen for use in these analyses (Table. 1).--

5. Please replace existing paragraph **0062** with the following paragraph:

--This example illustrates the amplicon produced during the amplification of VNTR locus Mtb-v1 with primer pairs SEQ ID NO: 1 and SEQ ID NO: 2.

5' Beginning location of VNTR: 55,533. (All accession numbers are according to the H37Rv *M. tuberculosis* sequence done by the Sanger Institute. <http://www.sanger.ac.uk/>)

GGCTCGGGCCTGCCGTCGGACATCTGGAAGGCAACCATGGACGGCGCCTTGAAGGG
CACGTCGAACGAGACTTTCCCCAAACCGACCGAGGTTCGGTGGTT
ATGCCGGTGTGCCGCCGCCGCCGCCGCCGCCGGAGGTACCACCTTCGCAG
ACCGTCATCCAGCCCACGGTCGAAATTGCGCCGGGGATTACCATCCCGATC
GGTCCCCCGACCACCATTACCCTGGCGCCACCGCCCCCGCCCCGCCCCGCTG
CGACTCCCACGCCGCCGCCGTGACCGGCGCGCTGTCCCAAAGCAGCAACATC
TCGCCACTTCCTTTGGCCGCCGATCTGCGGAGCGCCGATAACCGCGATTGCCC
CAGCCGCACCGACGTATTGGG (SEQ ID NO: 19)

FORWARD PRIMER 5' GTCGAACGAGACTTTCCCCAAACCGAC 3'
REVERSE PRIMER 5' GACCGTGGGCTGGATGACGGTCTC 3'

EXAMPLE 2--

6. Please replace existing paragraph 0063 with the following paragraph:

--This example illustrates the amplicon produced during the amplification of VNTR locus Mtb-v3 with primer pairs SEQ ID NO: 3 and SEQ ID NO: 4.

5' Beginning location of VNTR 241,464. (All accession numbers are according to the H37Rv *M. tuberculosis* sequence done by the Sanger Institute. <http://www.sanger.ac.uk/>)

5' GCGAGCCTACCGAAGATCGCGTGCATGCGTTCGGCGTGGACCGCACAGCA
CCTGGAGTTGGCGGCGCCGAGGGCCGAGATGGCAGGATGACCGATCGTTCG
GGGGCGGGAACTCCCAGGCCGCCGGGCGCGTCGCAAACCCGTCGCAAACC
CGTCGCAAACCGTAAGGAGTCATCCATGAAGACAGGCACCGCGACGACGCG

GCGCAGGCTGTTGGCAGTACTGATCGCCCTCGCGTTGCCGGGGGCGCCG
TTGCGCTGCTGGCCGAACCATCAGCGACCGGCGCGTCGGACCC,GTGCGCGGC
CAGCGAAGTGGCGAGGACGGTCGGTTCGGTCGCCAAGTCGATGGGCGACT 3' (SEQ
ID NO: 20)

FORWARD PRIMER 5' GATGACGGATCGTCGGGGGCGGGAAC 3'
REVERSE PRIMER 5' GCAACGCGAGGGCGATCAGTACTGCCAACA 3'

EXAMPLE 3--

7. Please replace existing paragraph **0064** with the following paragraph:

--This example illustrates the amplicon produced during the amplification of VNTR locus
Mtb-v4 with primer pairs SEQ ID NO: 5 and SEQ ID NO: 6.

5' Beginning location of VNTR -241,464. (All accession numbers are according to the H37Rv
M. tuberculosis sequence done by the Sanger Institute. <http://www.sanger.ac.uk/>)

ATTCAGGCCGCGAATCCACTACCGATGATGACCACGCGATGGCGCCCGC
CGACGGCCGAGGGTTCACCAGATGAGAGCGTCATGGTCCTCCTTCAGTCTGG
TCGCTGTGGCGCAGCTACACAGTACGACTCCCGTCATGCCAACGGCGTAA
CTTTTTGTGGGCCTTGTGGGCCTTGTGGGCCTTGTGGGCCTTTGTGCGGC
CGCCTTCGGATCGGACGCTCGGGATGGCTGTTGGGCGCTGCGCAATCCCGC
GCTTCGATCAGGCAGCGTCCGGCAGTGCCATCAATGGCGGCCAGGTACACCT
CTCCGACGGCTCGACATCGCCGGCCCGGCAGTTACCTGCACCATGGCCGGGC
GATGCGGGAGCGGCTGCCGAAGGTCGGGCAGGTGTTTGCTGCCGGGGAAATC
GACTACCACATGTTTCAGACGTTGGTGTATCGCACCGATTTGATCACCGACCC
GCAGGTGTTGGCGCGGGTGGATGCCGAGCTGGCGCTGCGGGTGCGGGGCT (SEQ ID
NO: 21)

FORWARD PRIMER 5' GCTGTGGCGCAGCTACACAGTACGACTC 3'
REVERSE PRIMER 5' GATTGCGCAGCGCCCAACAGC 3'

EXAMPLE 4--

8. Please replace existing paragraph **0065** with the following paragraph:

--This example illustrates the amplicon produced during the amplification of VNTR locus Mtb-v5 with primer pairs SEQ ID NO: 7 and SEQ ID NO: 8.

5' Beginning location of VNTR 241,464. (All accession numbers are according to the H37Rv *M. tuberculosis* sequence done by the Sanger Institute. <http://www.sanger.ac.uk/>)

AGCCAGCAGAGCTTCGAGGAAGTGTCTGGCGCGCGCCGAAGGCTACGTGG
ACCAGGCGGTGGAGTTGACCCAGGAGGCGT[[I']]TGGGTACGGTCGCATCGCAG
ACCCGCGCGGTCTGGTGAGCGTGCCGCCAAGCTGGTCGGCATCGAGCTGCCTA
AGAAGGCTGCTCCGGCCAAGAAGGCCGCTCCGGCCAAGAAGGCCGCTCC
GGCCAAGAAGGCGGGCGGCCAAGAAGGCGCCCGCGAAGAAGGCGGCGGC
CAAGAAGGTCACCCAGAAGTAGTCGGGCTCCGAATCACCATCGACTCCGA
GTCGCCCACGGGGCGACTCGGAGTCGACGTGTTGGATGCAAACCGCATAGTC
TGAATGCGTGAGCCACCTCGTGGGTACCGTCATGCTGGTATTGCTGGTCGCCG
TCTTGGTGACAGCGGTGTACGCGTTTGTGCATGCTGCGTTGCAGCGGCCCGAT
GCCTATACCGCCGCCGACAA (SEQ ID NO: 22)

FORWARD PRIMER 5' GGAGGCGTTGGGTACGGTCGCATC 3'
REVERSE PRIMER 5' GATTCGGAGCCCGACTACTTCTGGGTGAC 3'

EXAMPLE 5--

9. Please replace existing paragraph **0066** with the following paragraph:

--This example illustrates the amplicon produced during the amplification of VNTR locus Mtb-v6 with primer pairs SEQ ID NO: 9 and SEQ ID NO: 10.

5' Beginning location of VNTR 1,112,923. (All accession numbers are according to the H37Rv *M. tuberculosis* sequence done by the Sanger Institute. <http://www.sanger.ac.uk/>)

GCAGACCGCAGGTGCCGACGAGCCGGACTACTTAGACGTCGATGTGGTC
GAAGAAGACTCGGAGGCGCTTCCGGTGGGGGCTGGCGCTGCGGTTCGGCGAGT
CCGCCGACGAGGCCGATGCCGAAGCTGCTGACGGAGTTGCGGGCCACGCC
GACCCGGAGGCCGACCCGGTTCGAATACGAATACGAATACGAATACGTCGA
GGACACCTGCGGTTTGGAGCTCGAGGAGGACGACCAGGAAGCGCCACCGAC
CGTCGCATCCGGCACGTACGGCGGCGCCGATTCGACACCAAGACCGCCGCC
GCGGTCAGCGCCCGCAAGTACACCTTCCGCAAACGTGCGTTGATCGTGATGG
CGGTGATCCTGGTTGGCTCTGCCGCCGCGGCCTTCGAGCTGACCCCGGTCTG
CGTGGTGGATCTGTGGTAGCGCCACCGGTGTGACGGTGCTCTACCTGGCATAT
TTGCGTCGGCAAACCCGCATCGAGGAGAAGGTGCGTCGGCGGCGGATGCAG
CGGATCGCGCGGGCGCGGCTCGGTGTAGAGAACACCCGTG (SEQ ID NO: 23)

FORWARD PRIMER 5' CGCCGACGAGGCCGATGCCGAAGC 3'
REVERSE PRIMER 5' CCGCGGCGGCAGAGCCAACCAGGAT 3'

EXAMPLE 6

This example illustrates the amplicon produced during the amplification of VNTR locus Mtb-v10. Beginning location of VNTR 2,604,155. (All accession numbers are according to the H37Rv *M. tuberculosis* sequence done by the Sanger Institute. <http://www.sanger.ac.uk/>)

ATCCTTGCAGGTGTTTCGGTGGTGACGGTCGTGTTGTCGGCGGCTTCCAGG
CGCAACCGGAGGCGCCAGCCCCACAATCAGTGCCAACAGTGCCAACAG
TGCCAACAGTGCCAGAATCGGGACGGCGCTACGCTGACGACGCACGTCACG
AGCTTAGCGAAACTGGGAATTTCCCCTACGTTTCATCAACGCCTCAGGTGT
CGATCCTAAAGCGCGGGTGCCGCCGGTATTCTTGCCCCAAATCGGTTCGGTT
GACACCCGATGCGGTTCGGCGAAGCCATCGGCATCGCGGCCGACGACATCCCG

ATGGCGGCACGCTGGATCGGCA.GCCGACCATGCTCGCTCATCGGCCAGCC (SEQ ID NO: 24)

FORWARD PRIMER 5' GGAGGCGCCCAGCCCCACAA 3'
REVERSE PRIMER 5' TCAGGTGTCGATCCTAAAGCGCGGGTG 3'

EXAMPLE 7--

10. Please replace existing paragraph 0068 with the following paragraph:

--This example illustrates the amplicon produced during the amplification of VNTR locus Mtb-v15 with primer pairs SEQ ID NO: 13 and SEQ ID NO: 14. Beginning location of VNTR 3,238,462. (All accession numbers are according to the H37Rv *M. tuberculosis* sequence done by the Sanger Institute. <http://www.sanger.ac.uk/>)

CCGCGCCGATCGGTTCCGTTGATCCGCGCTCCCCGCAGCCGCGCCGTCAGA
TCCGCGGGCCCGGCATCGTGGCGGCGCACAGCGCGGGGGTGGTGCACCCGA
ACCTCGGTGATGGTCCGGCCGGTCACGTGAGCCTGCAAGCCGCGCCGCACC
ACCTCGACTTCGGGCAGCTCGGGCATCCAGTGATGATCGCAAGCGCGGGCG
AAGCCGGGCGCAGCGGGTCATCACCATCGAACCAGTGATGATCGCAAGC
GCGGCGAAGCCGGGCGCAGCGGGTCATCACCATCGAACCAGTGATGAT
CGCAAGCGCGGCGAAGCCGGGCGCAGTCCCCCGCAAGCGGGAGGTGCC
CCCAGGTCATCACCATCGAACCAGTGATCATCGCAAGCGCGGCGAAGCC
GGGCGCAGTCCCCCCCCAAGCGGGAGGTGCCCCCAGGTCATCACCATCG
AACCAGTGATGATCGCAAGCGCGGCGAACC CGGCCGCAGTCCCCCGCA
AGCGCGGCAAAGCCGGCGCCCCCAGGTCATCACCATCAATCCAGTTAGGCG
GAGGTTTTGCCCCGGCATGGCGTTGTCGAGCACTTCCAGGGCTTTCCAAGCG
GCCGCCGCGGCTTTTTGCTCGGCTTCTTTTTTGGACCGGCCCACTCCTGAAC (SEQ ID NO: 25)

FORWARD PRIMER 5' GCGCCGCACCACCTCGACTT 3'
REVERSE PRIMER 5' GTTAGGCGGAGGTTTTGCCCG 3'

EXAMPLE 8--

11. Please replace existing paragraph 0069 with the following paragraph:

--This example illustrates the amplicon produced during the amplification of VNTR locus Mtb-v18 with primer pairs SEQ ID NO 15 and SEQ ID NO 16.

5' Beginning location of VNTR 4,227,024. (All accession numbers are according to the H37Rv *M. tuberculosis* sequence done by the Sanger Institute. <http://www.sanger.ac.uk/>)

CGGGCTATCACCGGTTTCATCTTCTGGGCCTATGGCCGCAACGGGGAGAGCG
ATCCTCGGGTACTCGAGGCCATCCAAGTCCTCCGTATCCGCTATATCCTGACC
AGCACTCCGACGGTGCGGGGGTTTGCCGTGCCGGACGGACTAGTGTCGTTAG
AGACATCGAGGTCGTGGGCGAAGATCTACGACAACGGCGAGGCCCGAATC
TACGAATGGCGCGGCACTGCCGCAGCAACACACTCCTAGAAGGTGCGTAAG
AGGATGGTGATTGGATTGAGTACCGGCAGCGACGACGACGACGTCGAGGTCA
TCGGCGGCGTCGACCCGCGGCTGATAGCGGTGCAGGAGAACGACTCCGACG
AGTCGTCGCTGACCGACCTGGTCGAGCAGCCCGCCAAGGTGATGCGCATCGG
'CACCATGATCAAGCAACTGCTCGAGGAGGTTTCGCGCCGCCCCACTCGACGAA
GCCAGCCGCAATCGGCTACGCGATATCCACGCCACCAGCATCCGCGAACTC
GAAGATGGTCTGGCCCCGGAAGTGC GCGAGGAGCTCGACCGGCTTACCCTGC
CGTTCAACGAGGACGCCGTGCCCTCGGACGCCGAGTTGCGCATTGCCAGGC
ACAGCTGGTCGGCTGGCTGGAAGGGCTGTTCCACGGCATCCAAA (SEQ ID NO: 26)

FORWARD PRIMER 5' ACAACGGCGAGGCCCGAATCTACGAA 3'
REVERSE PRIMER 5' ATAGGTGCGGTGGTCGTAGGCGC 3'

EXAMPLE 9--

12. Please replace existing paragraph 0070 with the following paragraph:

This example illustrates the amplicon produced during the amplification of VNTR locus Mtb-v20 with primer pairs SEQ ID NO 17 and SEQ ID NO 18. Beginning location of VNTR 23,693. (All accession numbers are according to the H37Rv *M. tuberculosis* sequence done by the Sanger Institute. <http://www.sanger.ac.uk/>)

AGCGTATCCCGGGACCCGCGGGGATTCGGTGTAGCGGGTGTAGTCAGCGCC
GCCGCCATAGTCTTGCCGGCCGTATGTCGTGGCGCCTTGCTGGTAGCCCTGGT
CGTAACCGCCTTGGTCGGGGTAAGCCGGTCGTTGCTCGGGCGGGCCCGGAG
GGCCAGAGGGGCACATAGCTGCCCTCCTCGTGGCGAGCCGGGGCCACGCCCCT
ACTCCCCGTACCCGCCGTAGCCGGGCTGGCCGCCACCGGGTGAAGGGCCGTA
GCCGCCGCTTTGGCGATAGCCCTGGTCGTAGCCGGGAGCGCCGTAGCCGGCA
GCCGGGCGGGAGAAACAGGAGGGCGTTGCTCGTAGGGCGGCGGATAGCCC
CCCTGCCCTTGGTCGGGGTAGCCTCGACCCTGGTCCTGGTACCCGCGCTGGTC
GGGGTAGCCGCGTTGCTCGGGGTAACCGCGTTGCTCGGGGTAACCGCCC
TGGTCGGGGTACCCGATTTGCTCGGGGTAGTCGCCCTGGTCCGGGTGGCGCG
GGCGTGGGTAGCCCGGCTGGGGCGGGTAGCCGCCCGTCTCGGGTGGATACCC
CCCGCGGGGGTCAGATCCGCCTTGCGGATCCGGGGCCACCACGCGGATCCTCT
TGCGGACGCGCATAGCGGTCGTCGTAATACTCGTCGGGACGCCCCTGCCCCT
GACCGCCACGGTAGCTCGAATTGTCACCTCATTGGTGCTACTCCTGGTTCTG
CGCCAAACGCGTGTTTGATTGTGGCCGGGCGCAATCGATGACCGGCGG (SEQ ID
NO: 27)

FORWARD PRIMER 5' CCCGGAGGGCCAGAGGGCACATAGC 3'
REVERSE PRIMER 5' GTCACCTCATTGGTGCTACTCCTGGTTCTGCGCCA 3'--

13. Please replace the existing **Sequence Listing** with the Sequence Listing appended to the end of this Response.

14. Please add **pages 28 and 29** to the specification (appended to the end of this response).

15. Please replace the existing **Abstract** with the following Abstract:

(On separate sheet in accordance with 37 C.F.R. 1.72; see next page).